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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/685,837	SEIBLER ET AL.	
	Examiner	Art Unit	
	Anoop Singh	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-30* is/are pending in the application.

4a) Of the above claim(s) *28 and 29* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-27 and 30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☒ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *03/30/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's election with traverse of the invention of group IV (claim 27) filled Oct. 24, 2005 is acknowledged. The traversal is on the grounds(s) that Examiner has not set forth convincing argument that the search and examination of group I and other groups such as II, III, V and VI necessarily represents an undue burden for the examiner and that examination of studying method of knock down in vertebrate, tissue and cell along with elected group directed would not require separate searches for prior art. Applicant argument of examining method for gene knock down in a vertebrate (group 1) with elected group were found persuasive, therefore invention of group I and IV directed to vertebrate and method of gene knock down in a vertebrate are rejoined for the examination purposes. However, Applicant argument for examining other groups (II, III, V, VI) and that examiner must show a burden of searching by separate classification are not persuasive because classification is very broad grouping of inventions and very different and distinct inventions are present in particular class/subclass. Furthermore, the examination of the invention of different method and/or composition groups would require undue search burden because for examining a method claim for knock down in a cell/tissue is only one limitation and Examiner has to consider the method steps and perform searches. For example, method of introducing a construct to a cell or a tissue would be different from that of a vertebrate and thus require separate searches and search would be an undue burden since method and factors affecting these distinct

steps would have to be considered. Additionally, the different inventions have different status in the art because they are drawn to different structure and functions.
The requirement is still deemed proper and is therefore made FINAL.

Accordingly, a method for gene knock down in a vertebrate and vertebrate having stable integration at Polymerase II dependent locus, an expression vector comprising an shRNA construct under control of a ubiquitous promoter will be examined in the instant application.

2. Groups II-III and V-VI have been have been withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on October 24, 2005.

3. Claims 1-27 and 30 are under consideration.

It is noted that the claims 1-27 and 30 of groups I and IV are included in multiple groups because they encompass the inventions of these groups. However these claims will be examined to the extent they encompass the invention of the elected group, a vertebrate and method of gene knockdown in a vertebrate having stably integrated, preferably at a polymerase II dependent locus of the vertebrate, an expression vector comprising a short hairpin RNA (shRNA) construct under control of a ubiquitous promoter.

Claim Objections

4. Claims 1-27 are objected to because of the following informalities: Claims 1-26 are rejoined with elected group of invention. Claims 1-27 continue to depend in part to claims that are withdrawn and should be rewritten in independent form to recite elected invention. Appropriate correction is required.

Priority

5. Acknowledgment is made of applicant's claim for foreign priority based on an application filed in Europe on 10/17/2002. It is noted, however, that applicant has not filed a certified copy of the priority application as required by 35 U.S.C. 119(b).

Claim Rejections - 35 USC § 101

6. 35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

7. Claims 1-27 rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter.

The claim is directed to a "genetically modified vertebrate". As written in view of the teaching of the instant specification the claimed vertebrate reads on transgenic

human being. A human being or human embryo is non-statutory subject matter. See 1077 O.G. 24 April 21, 1987.

> (Animals - Patentability, 1077 O.G. 24, April 21, 1987) that the Patent and Trademark Office would now consider non naturally occurring, nonhuman multi cellular living organisms, including animals, to be patentable subject matter within the scope of 35 U.S.C. 101.If the broadest reasonable interpretation of the claimed invention as a whole encompasses a human being, then a rejection under 35 U.S.C. 101 must be made indicating that the claimed invention is directed to nonstatutory subject matter. Furthermore, the claimed invention must be examined with regard to all issues pertinent to patentability, and any applicable rejections under 35 U.S.C. 102, 103, or 112 must also be made.)

Claim Rejections - 35 USC § 112

8. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

9. Claims 2-4, 6, 9, 12, 14-15, 18 and 27 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The claim 27 is vague and indefinite because it uses the term “preferably at Polymerase II dependent locus” of vertebrate because the term is not defined and subjective depending on artisan, thus the meets and bounds of the claim cannot be determined. For examination purposes it is not considered as a limitation.

Regarding claims 6, 9 and 12 the phrase "etc." renders the claim(s) indefinite because the claim(s) include(s) elements not actually disclosed (those encompassed by "etc"), thereby rendering the scope of the claim(s) unascertainable. The meets and bounds of the claimed invention cannot be determined.

The term "Suitable" in claims 2-4, 15, 18 is a relative term which renders the claim indefinite. The term "suitable" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. The meets and bounds of the claimed invention cannot be determined.

Claim Rejections - 35 USC § 112

10. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

11. Claims 1-27 rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of gene knockdown in a mouse genome at the *rosa26* locus, said method comprising introducing a shRNA and reporter constructs in mouse embryonic stem cell by homologous recombination, wherein said shRNA and reporter constructs comprises a gene encoding Renilla (Rluc) and luciferase (Fluc) along with an adenovirus splice acceptor sequence and polyadenlylation signal placed downstream of the endogenous promoter of rosa26, and Fluc specific shRNA expressed under the control of H1 and U6 promoter and terminated by five thymidines; and microinjecting said mouse embryonic stem cell into mouse diploid blastocysts; and implanting the blastocysts comprising the mouse embryonic stem cell into pseudo pregnant mouse; allowing the resulting pregnant

mouse to deliver viable chimeric offspring and a transgenic mouse produced by said method, wherein said transgenic mouse exhibits ~90% reduced luciferase activity in liver, heart, brain and muscle, does not reasonably provide enablement for the method of gene knockdown in any vertebrate using any promoter integrated at any locus with any shRNA sequence. The instant claim encompasses genetic alteration of any living organism, including humans. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claim 1 encompasses a method for constitutive and/or inducible gene knock down in a vertebrate comprising an shRNA construct under control of a ubiquitous promoter into the genome of the vertebrate. The dependent claims 2-5 limit the expression vector being suitable for stable integration in genome and contains homologous sequence for integration at defined locus through homologous recombination at polymerase II dependent locus selected from list of a group consisting of rosa26, collagen, RNA polymerase, actin and HPRT locus. Claims 6-14 encompass the expression vector of claim 1, which further contains functional sequences and group of promoters, which is either constitutive or inducible. The inducible promoter expression is a promoter consisting an operator sequence and the vertebrate of claim 1 is non-human vertebrate, which is further limited to either mouse or fish.

Claims 15-19 encompass method of claim 1 wherein the vector is a either Pol III or Pol II dependent promoter driven shRNA construct suitable for integration into a Pol II

dependent locus. Subsequent claim limit the Pol III promoter being either constitutive or inducible H1 or U6 promoter while Pol II promoter being inducible CMV promoter.

Claims 20-27 encompass method of claim 1 which describes the shRNA segment comprising a stop and or polyadenylation sequence which is integrated at polymerase dependent locus of the vertebrate and ES cell of the vertebrate.

The application as filed is not enabling for the invention commensurate with the full scope of the claims because art of gene knockdown by shRNA in any species using any locus and any vector is unpredictable as has been recognized by the art of skill and therefore require undue experimentation. As will be shown below, these broad aspects as well as limitations were not enabled for the claimed invention commensurate with the full scope of the claims at the time of filling of this application because neither the specification nor the art of record taught sufficient guidance to practice the claimed invention commensurate with the scope of the claim.

In determining whether Applicant's claims are enabled, it must be found that one of skill in the art at the time of invention by applicant would not have had to perform "undue experimentation" to make and/or use the invention claimed. Such a determination is not a simple factual consideration, but is a conclusion reached by weighing at least eight factors as set forth in In re Wands, 858 F.2d at 737, 8 USPQ 1400, 2d at 1404. Such factors are: (1) The breadth of the claims; (2) The nature of the invention; (3) The state of the art; (4) The level of one of ordinary skill in the art; (5) The level of predictability in the art; (6) The amount of direction and guidance provided by

Applicant; (7) The existence of working examples; and (8) The quantity of experimentation needed to make and/or use the invention.

These factors will be analyzed, in turn, to demonstrate that one of ordinary skill in the art would have had to perform "undue experimentation" to make and/or use the invention and therefore, applicant's claims are not enabled commensurate with the full scope of the claims.

Furthermore, USPTO does not have laboratory facilities to test if an invention will function as claimed when working example are not disclosed in the specification, therefore enablement issues are raised and discussed based on the state of knowledge pertinent to an art at the time of the invention, therefore, skepticism raised in enablement rejections are those raised in the art by artisan of expertise.

Claims 1-27 are broad in scope. The following paragraph will outline the full scope of the claims:

Claimed invention recites a method of gene knockdown in a vertebrate having stably integrated, at different locus of the vertebrate, an expression vector comprising a short hairpin RNA (shRNA) construct under control of any ubiquitous promoter.

Since these claims are broad in scope, encompassing any vertebrate having stably integrated at any locus subsequently limiting to few by shRNA construct under control of any ubiquitous promoter, the disclosure provided by the applicant, in view of prior art, must encompass a wide area of knowledge to a reasonably comprehensive extent. In other word each of those aspect considered broad must be shown to a reasonable extent so that one of the ordinary skill in the art at the time of invention by

applicant, would be able to practice the invention without any undue burden being on such Artisan.

The specification broadly discloses the progression of RNA interference technology over the years (pp. 1,2) and describes role of shRNA-mediated gene silencing in transgenic mice and rats. The invention is based in part of a method of using a expression vector comprising a short hairpin RNA construct under the control of ubiquitous promoter for gene knock down in a living organism (pp 3). Pages 4-6 provide short description of figures. Pages 7-14 of the specification disclose definition of terms, general description of ubiquitous promoter, expression vector and a general description of different shRNA sequence in tabular form. Pages 15-17 broadly discusses preferred embodiments of the method steps comprising of generating shRNA and construct for cell culture, luciferase measurement assay and generation of chimeric mice. Example 1: of specification teaches the firefly luciferase gene along with a splice acceptor sequence is inserted into first allele of rosa26 locus by homologous recombination in ES cells while shRNA and Renilla luciferase gene is inserted into second allele of rosa 26. Figure 7 shows the expression of the firefly luciferase in presence and absence of shRNA expression cassette. Example 2 shows shRNA expression cassette under control of U6 promoter containing tet operator sequence and a Renilla luciferase gene is inserted into first allele of rosa26 locus (figure 8 and 12), while the luciferase gene with a promoter and a tet repressor expression cassette is introduced into the second allele in ES cells. Luciferece activity is shown in presence and absence of doxycycline. Example 3 page 19 describes that NIH3T3 cells are transiently transfected with

construct expressing the luciferase and tet repressor gene together with the shRNA construct containing tet operator sequence. Figure 11 shows the expression of luciferase in presence and absence of doxycycline. The specification discloses doxycycline inducible shRNA expression resulted ~80% inhibition in firefly luciferase activity in cells. Example 4 pages 19 show chimeric mice from rosa26/U6 and H1-ShRNA transgene. The data shows shRNA construct under the control of both U6 and H1 effectively repressed the firefly luciferase activity inmost organs (Figure 13B).

However, such broad disclosure does not demonstrate the information required by the Artisan to reasonably make and use any vertebrate with an expression vector comprising any short hairpin RNA (shRNA) construct under control of a any ubiquitous promoter integrated at any locus. The specification does not provide any specific guidance with how any vertebrate could be genetically modified. In fact, Applicant's examples only describe a method to knock down expression of luciferase using shRNA technology however, none of the examples demonstrate any specific *in vivo* phenotype that correlate to any condition in the instant application. At the time of the invention, although many of the methods are routine, neither the art of record nor the specification teaches how to practice the claimed invention for all different type vertebrate and how ES cells from different species are going to be obtained and cultured. An artisan of skill would have required undue experimentation to practice the claimed invention because the method as recited involves culture of multiple ES cells. An artisan would have to carry out extensive experimentation to make and use the invention, and such experimentation would have been undue because of the art of making any transgenic

mice without any specific phenotype were unpredictable and specification fails to provide any guidance as to how the claimed method would have been practiced.

Claims 1-27 are directed to method of making vertebrate whose genome is modified and requires embryonic stem cell for stable integration of the construct. The art at the time of filing further held that transgenic technology was not predictable for any species other than mouse. Since the specification discloses using mouse ES cells to produce transgenic mice via homologous recombination of targeting vectors in the ES cells, ES cells from various species are required to produce various vertebrates. However, Houdebine, 1994 (Journal of Biotechnology, Vol., 34, pp 269-287) describes that although ES cells can be used to generate transgenic animals, but this approach remains restricted to mice, ES cells from other species are not presently available (pp 279). Furthermore Mullin et al also point that non-mouse ES cell capable of providing germ line chimeras were not available (Mullins et al., Journal of Clinical Investigation, 1996, pp 1557, 1st paragraph). Campbell and Wilmut (1997, Therigenology) acknowledges report of ES-like cells in number of species, but also emphasize that there are no report of any cell line that contribute to germ line in any species other than mouse (pp 65; 2nd paragraph). Thus the state of the art is such that ES cell technology is generally limited to the mouse system and that only putative ES cells exist for other species (Moreadith et al., J. Mol. Med., 1997 p214, abstract). Therefore, at the time of filing of this application, method of gene knockdown in any vertebrate could not be accomplished for any species other than mouse.

The specification fails to provide sufficient guidance to make non-human transgenic other than mice by teaching obtaining ES cells in species other than mice. The specification also fails to provide sufficient guidance to make transgenic mice using stem cell other than ES cells. The specification does not teach how to make knock down vertebrate by shRNA for any other species other than mice or correlate making mice to making knockout for any other species. Therefore, the claims should be limited to mice and method of making such mice as discussed in the office action.

Claim 27 recites a vertebrate with an expression vector comprising a short hairpin RNA (shRNA) construct under control of a ubiquitous promoter integrated at a polymerase II dependent locus. However the specification as filed does not provide any specific information about resulting phenotype of the claimed invention. It is noted that the specification merely recites the luciferase activity in different organ, however it dose not provide any specific information for practicing the claimed invention commensurate with the full scope of the claim. The specification teaches method of making a transgenic mice-using shRNA. However, it is noted that art recognizes that the resultant phenotype, when producing knockdown mice, is exceedingly unpredictable. For example, Leonard (Immunological Reviews, 1995, 148: 98-114) discloses mice with disruption in the gc gene that was intended to be a model for X-linked severe combined immunodeficiency (XCIDS), but displays a variety of unexpected traits (Abstract). These knockout mice were expected to have thymocytes with decreased proliferation in response to stimulation with antibodies, but the thymocytes proliferated normally (pp 105, line 7). Griffiths (Microscopy Research and Technique 1998, 41: 344-358) taught

that, despite a known role for the PLP gene based on spontaneous mutations in the gene, the knockout mouse failed to display any of the expected phenotype (pp 350, last paragraph). Thus at the time of filing, the resulting phenotype of a knockout was considered unpredictable. Furthermore, there are reports of X-linked inactivation in the *hprt* locus. This has been shown to cause complications in breeding the animal. Although *hprt* locus has been used to target several different transgenic DNA vectors, however not all of these vectors have shown the pattern of expression expected from the choice of promoter used to drive the trangene in these vectors (Hatada et al., 1999; J Biol Chem, 274, 948-955; entire article).

Note that mere capability to perform gene transfer in a mouse is not enabling because a desired phenotype cannot be predictably achieved by simply introducing transgene construct as recited in the claims. Holschneider et al. (Int J Devl Neuroscience, 2000, 18: 615-618) state that single genes are often essential in a number of different physiological processes. Hence deletion of an individual gene may prove so drastic or so widespread as to create an amalgam of phenotypes whose interpretation becomes confounded by the interaction of various new physiologic changes (pp 615). Holschneider et al discuss various factors that contribute to the resulting phenotype of transgenic mice, including compensatory system that may be activated to mask the resulting phenotype; these compensatory changes may be due to differential expression of another gene, which may be regulated by the downstream product of the deleted gene.

It is not apparent how skilled artisan without any undue experimentation, practices method as contemplated by the instant claims particularly given the unpredictability of using different loci, construct and shRNA sequence as whole and unpredictability expressed in the art.

The specification further describes expression vector is also suitable for transient integration (pp7; 4th paragraph). The unpredictability of attenuating /inhibiting expression of a target gene in cell by RNAi is evident in prior and post filing art. While it is recognized that introduction of dsRNA that is targeted to a specific gene may result in attenuation /inhibition of the targeted gene, the degree of attenuation and length of the time attenuation is achieved in not predictable. Caplen et al (Gene 2000, vol. 252, 95-105) provide evidence of the unpredictability of dsRNA attenuation /inhibition of targeted gene in vertebrate cell in vitro. Transient transfection of dsRNA to the βgal trasngene into 293 and BHK31 cells resulted either in no effect or a non-specific decrease in gene expression (pp102; Figure 7 A and B). Prawitt et al (Cytogenet Genome Res. 105 (2-4), 412-421, 2004) state stable expression of shRNA may be probably less harsh and may induce less non-specific effects as compare to transient trasnfection. The post-filing art of Prawitt et al. (Cytogenet Genome Res. 105 (2-4), 412-421, 2004) reviews the state of the art with regard to use of RNAi in generating knock down mice.

Prawitt et al. describe that recent studies have shown that expression of shRNA in mammalian cell induced target gene for **interferon pathways** (pp419, column 1, 2nd paragraph, references therein). In view of these studies, Prawitt et al stressed the importance of interpreting the RNAi effects both in tissue culture as well as in mouse.

Furthermore, Prawitt et al evinces an optimistic outlook for tetracycline inducible system in generating inducible knock down mouse, but also acknowledges that the art is still unpredictable by stating "it remains to be proven if doxycycline can be used to study as graded knock down phenotype in mouse" (pp 419; 2nd column).

In view of lack of teaching or guidance provided by the specification with regard to an enabled method for gene knockdown in any vertebrate comprising a disruption in gene using any shRNA, construct comprising different constitutive or inducible promoter, locus and shRNA sequence and the lack of teaching or guidance provided by the specification to overcome the art recognized unpredictability of disruption of a particular gene, promoter and locus and the resulting phenotype and absence of any correlation between disruption and its phenotype, for the specific reason cited above in the office action. It would require undue experimentation for an Artisan to make and use the claimed invention and/or working examples demonstrating the same, such invention as claimed by the applicant is not enabled for the claimed inventions commensurate with the full scope of the claims.

12. Claim 1-27 rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter, which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claimed invention encompasses any method of gene knockdown in any vertebrate having stable integration preferably at different locus

with an expression vector comprising an shRNA construct under control of any ubiquitous promoter.

When claims are analyzed in light of the specification, instant invention encompasses any vertebrate, any locus and under control of any ubiquitous promoter. In analyzing whether the written description requirement is met for the genus claim, it is first determined whether a representative number of species have been described by their complete structure. In the instant case the specification merely recites a general description of the methodology to gene knockdown of luciferase gene. The specification does not provide any disclosure as to what would have been the structure of the representative number of the species of the claimed broad genus that encompasses any vertebrate, any locus, promoter and many shRNA sequence as disclosed in specification.

Next, then it is determined whether a representative number of species have been sufficiently described by other relevant identifying characteristics, specific features and functional attributes that would distinguish different members of the claimed genus. In the instant case, the specification merely shows the luciferase activity in mice. The specification,does not teach the structure and identifying characteristics of a sufficient number of representative species of different locus or ubiquitous promoter or all claimed species.

In conclusion, this limited information is not deemed sufficient to reasonably convey one skilled in art that Applicant was in possession of the claimed broad genus at

the time the application was filed. Thus it is concluded that the written description requirement is not satisfied for the claimed broad inventions.

Claim Rejections - 35 USC § 102

13. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

The changes made to 35 U.S.C. 102(e) by the American Inventors Protection Act of 1999 (AIPA) and the Intellectual Property and High Technology Technical Amendments Act of 2002 do not apply when the reference is a U.S. patent resulting directly or indirectly from an international application filed before November 29, 2000. Therefore, the prior art date of the reference is determined under 35 U.S.C. 102(e) prior to the amendment by the AIPA (pre-AIPA 35 U.S.C. 102(e)).

14. Claim 1-2, 7-9, 13-14, 27 and 30 rejected under 35 U.S.C. 102(a) as being anticipated by McCaffrey et al., (Nature, 2002 Vol. 418, 38-39; IDS).

Claims are directed to a method of making **vertebrate** having stably integrated, preferably at a polymerase II dependent locus of the vertebrate, an expression vector comprising a short hairpin RNA (shRNA) construct under control of a ubiquitous promoter.

McCaffrey et al teach functional shRNA expression in vivo from DNA template using RNA polymerase III promoters in inducing luciferase gene suppression (pp38, Figure 1 C-D and pp39 2nd paragraph) in mice.

Accordingly, McCaffrey et al., anticipate claims 1-2, 7-9, 13-14, 27 and 30.

15. Claim 30 rejected under 35 U.S.C. 102(a) as being anticipated by Paddison et al (Gene & Development, 2002, 16, 948-958).

Claim 30 is drawn to an expression vector comprising a short hairpin RNA (shRNA) construct under control of a ubiquitous promoter.

Paddison et al teach a linker/terminator oligonucleotide set bearing the U6 terminator sequence and linker ends cloned into a promoter construct, resulting in a U6 cassette. Paddison et al disclose a blunt ended dsDNA oligonucleotide encoding shRNA with 19-29 bases of homology to the targeted gene that is ligated to produce shRNA expression cassette, which is preceded by Pol III terminator (pp 955, Figure 4).

Accordingly, Paddison et al., anticipate claim 30.

16. Claims 1-2, 7-11, 13-14, 24-27 and 30 rejected under 35 U.S.C. 102(e) as being anticipated by Beach et al. (US patent Publication no. 2003/0084471, publication date 5/1/2003; filing date 1/22/2002).

Claim 1 is directed to gene knock down in a vertebrate that comprises an expression vector comprising a short hairpin construct under the control of ubiquitous promoter. Claim 6-7 limit expression vector further contains functional sequence selected from the group consisting of splice acceptor sequence, polyadneylation sites, selectable marker and the promoter of claim 1 is selected from a group comprising Polymerase I, II and III promoter which is further limited to either Polymerase II or II promoter. Claim 9 limits the ubiquitous promoter to a group consisting of a list of promoters. Claim 10 and 11 further limit the promoter of claim 1 to either constitutive or inducible promoter. Claim 13 and 14 limits the vertebrate as non-human vertebrate that is either mouse or a fish. Claim 15 is directed to method of claim 1 wherein the expression vector is Pol III dependent promoter suitable for integration at Pol II dependent locus. Claims 16 and 17 limits the promoter to be either constitutive or inducible H1 or U6.

Beach et al disclose that the double-stranded structure may be formed by a single self-complementary RNA strand or two complementary RNA strands. RNA duplex formation may be initiated either inside or outside the cell. Inhibition is sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition (pp4, paragraph 52). Beach et al teach the length of the

dsRNA is at least **20**, **21** or **22** nucleotides in length, e.g., corresponding in size to RNA products produced by Dicer-dependent cleavage. In certain embodiments, the dsRNA construct is at least 25, 50, 100, 200, 300 or 400 bases (pp13, paragraph 16). Beach et al disclose that the dsRNA construct may be synthesized either in vivo or in vitro. RNA can be derived from an **expression construct** (pp 13,14; paragraph 168). The invention also discloses strategy for stable expression of dsRNA in cultured mammalian cells (Figure 27, paragraph 78). Beach et al disclose generating several types of short dsRNAs corresponding to the coding region of **firefly** or **Renilla luciferase** (pp22; paragraph 246). Beach et al demonstrates that **short hairpins** encoded on a plasmid are effective in suppressing luciferase gene expression (Figure 42) in vivo. **DNA oligonucleotides** encoding **29** nucleotide hairpins corresponding to firefly luciferase were inserted into a **vector** containing the **U6** promoter. Beach further disclose that one of skill can choose from amongst a range of vectors to either transiently or **stably express** an short hairpin. Beach et al also disclose non-limiting examples of vectors and strategies to **stably express** short dsRNAs using U6 and H1 promoters (pp23; paragraph 252; Figures. 43-45). Beach et al teach and claim a **non-human transgenic mammal** having germline and/or somatic cells comprising a transgene encoding a dsRNA construct (pp 26, claim 28 and pp 2 paragraph 52) that includes **rodent** (pp12, paragraph 154). Beach et al demonstrates that a short hairpin is highly effective in specifically suppressing gene expression of firefly or Renilla luciferase (Example 6).

Accordingly, Beach et al anticipate claims 1-2, 7-11, 13-16, 20-27 and 30.

17. No Claims allowed.

18. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anoop Singh whose telephone number is (571) 272-3306. The examiner can normally be reached on 8:30AM-5:00PM. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272- 0735. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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